Mutations in the Tyrosine Kinase Domain of the Epidermal Growth Factor Receptor in Non–Small Cell Lung Cancer

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ABSTRACT

We evaluated somatic genetic alterations in the kinase domain of the *EGFR* gene in the tumors of 219 non–small cell lung cancer patients of primarily Caucasian and African American origins. We identified 26 patients (12%) whose tumors had a mutation in the *EGFR* gene, and 11 (5%) patients carried novel genomic variations consistent with germ-line polymorphisms. All but one mutation were identified in Caucasian patients affected with adenocarcinoma. *EGFR* mutations were more frequent in women and in nonsmokers, but a significant portion of the affected patients were men (12 of 26) and current or past smokers accounted for half of the patients affected (13 of 26). Screening subjects with *EGFR* mutations may identify patients whose tumors could respond to targeted therapy using tyrosine kinase inhibitors.

INTRODUCTION

Non–small cell lung cancer (NSCLC) accounts for more than 85% of all primary lung cancer cases and is the leading cause of death from cancer in the United States and worldwide (1, 2). Although anatomic resection of the primary tumor remains the most effective treatment for NSCLC, chemotherapy has also proven to be marginally successful (3). In an effort to develop more specific and effective therapies, the epidermal growth factor family of receptors has been identified as a potential target for NSCLC therapy (4). Epidermal growth factor receptor (EGFR) is overexpressed in a significant portion of NSCLCs (5, 6). An inhibitor of the EGFR tyrosine kinase, gefitinib (Iressa), was recently approved for the treatment of NSCLC (7–9), although clinical trials have revealed significant variability in the response to gefitinib (10–12).

A potential molecular mechanism underlying this variable clinical response was indicated by recent reports showing that the presence of an *EGFR* gene mutation correlates with clinical response to gefitinib (13–15). Jointly, these reports showed that *EGFR* gene mutation was present in the tumors of a majority of the patients who responded to gefitinib, a kinase inhibitor, and most of the affected patients were females and nonsmokers with adenocarcinoma or bronchioloalveolar carcinoma. In the study by Paez et al., the Japanese population seemed to have a much higher rate of *EGFR* gene mutation (15 of 58, 26%) compared with a US cohort (1 of 61, 2%). In contrast, *EGFR* gene mutations were infrequent among NSCLC patients who were untreated or did not response to gefitinib. When 95 extrapulmonary cancers and 108 cancer-derived cell lines were analyzed, no *EGFR* gene mutation was found (13). Similarly, the study by Pao et al. (15) supported the notion that the kinase domain of the *EGFR* gene was often mutated in patients who showed clinical response to kinase inhibitor treatment and were more common in female adenocarcinoma patients with low exposures to cigarette smoking.

In combination, the available data indicate that *EGFR* mutations may be associated with specific demographic, exposure, histologic, and ethnic or geographic features among lung cancer patients. We therefore evaluated somatic genetic alterations in the kinase domain of the *EGFR* gene in the tumors of 219 NSCLC patients from three study cohorts that were designed to assess cigarette smoking, age of onset, and gender and race contributions to lung cancer. Our aim was to assess the overall frequency of *EGFR* mutations in patients with different epidemiologic and etiologic backgrounds.

MATERIALS AND METHODS

Tissue Samples and DNA Extraction. Tumor and matched normal tissues from 219 patients diagnosed with primary lung cancer were obtained from the University of Maryland Medical Center (127 samples), the Mayo Clinic in Minnesota (64 samples), and the University of Milan, Italy (28 samples). All patient samples were collected or tested with informed consent, as approved by their respective institutional review boards. Clinical variables for each patient were obtained from the medical record. There is no known history of kinase inhibitor treatment for any of the patients. Representative sections from tissue used for DNA extraction were stained with H&E to determine tumor histologic subtype and assess tumor content. Fresh frozen tissues from the tumors were grossly dissected to ensure that specimens contained at least 50% tumor cells. Approximately 10 sections of 20-mm thickness were collected from normal and tumor samples and placed in either

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Trizol reagents (Invitrogen, Carlsbad, CA) or 1% SDS/proteinase K (10 mg/mL) at 58°C overnight. For samples placed in Trizol, DNA was extracted from the organic phase after RNA extraction following the protocol from the manufacturer. All samples were then subjected to phenol-chloroform extraction and ethanol precipitation. Clinical information was available from all 219 patients and Table 1 summarizes the demographic and clinical data of the study cohorts.

PCR and Sequencing Methods for Genomic DNA. We PCR-amplified and sequenced exons 18 to 21 of the *EGFR* gene using primers as described (13). The PCR reaction was carried out using 10 ng of total genomic DNA, incubated at 95°C for 10 minutes, then 39 cycles of 94°C for 30 seconds, 56°C for 30 seconds, 72°C for 1 minute, and final extension at 72°C for 10 minutes in a mixture containing 1× PCR Gold buffer (Roche, Basel, Switzerland) with 2 mmol/L $MgCl_2$, 0.8 mmol/L deoxynucleotide triphosphates, and 10 pmol of each primer. The amplified products (8 μL) were mixed and treated with 0.5 unit of shrimp alkaline phosphatase and 4 units of exonuclease I and incubated at 37°C for 60 minutes, and then heated at 72°C for 10 minutes to inactivate the enzymes. Sequencing conditions for *EGFR* were 94°C for 10 seconds, 50°C for 10 seconds, and 60°C for 2 minutes for 24 cycles. The PCR product of exon 19 was analyzed on an ABI PRISM 3730 (Applied Biosystems, Foster City, CA) automatic sequencer to identify the presence of the shorter deleted allele, and PCR products for exons 18, 20, and 21 were directly sequenced in both directions for mutations in primary tumors. All alterations were confirmed to be somatic or germ line by sequencing the paired normal samples except in four cases (57767M, 1061I, 78519M, and 85978M) in which the normal sample could not be amplified by PCR.

Statistical Analysis. Differences in the characteristics of lung cancer patients with or without *EGFR* mutations were compared by χ^2 test or Fisher's exact tests when 20% of the expected counts were less than 5. The 95% confidence intervals (95% CI) for odds ratios and frequencies were calculated as exact confidence intervals (SAS version 8.1; SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Of the 219 tumors we analyzed, 127 samples were from the University of Maryland Medical Center and consisted of 87 and 40 patients of Caucasian and African American descent, respectively. The patients from the Mayo Clinic in Minnesota (64 cases; ref. 16) had one patient each of African American and Hispanic descent and the University of Milan, Italy (28 cases) had patients with Caucasian descent only. Table 1 summarizes the patient characteristics for all patients as well as the frequency of *EGFR* gene mutations in each category. Overall, exons 18, 19, and 20 were successfully analyzed in 218, 217, and 210 patients, respectively, and exon 21 was analyzed in all 219 patients. When limiting to Caucasians, the occurrence of the *EGFR* gene mutation was not statistically different among the three groups ($P = 0.074$). When limiting only to adenocarcinomas, the mutation frequencies in these populations were also similar ($P = 0.353$). Therefore, these three cohorts were considered jointly when considering the frequency of *EGFR* gene mutations. Among the cases analyzed, 26 tumors (11.9% overall) had a mutation in the *EGFR* gene, and 11 patients (5.0% of total) carried low frequency genomic variants consistent with germ-line polymorphisms. Age at diagnosis of lung cancer, before or after 65 years, did not modify the frequency of *EGFR* mutations ($P = 0.254$). Among the 26 patients whose tumors had *EGFR* gene mutations, 14 (54%) patients were women and 12 (46%) were men ($P = 0.094$). Twenty-five of the 177 Caucasian

Table 1 Clinical characteristics of lung cancer patients

					EGFR status	
	Maryland	Mayo	Italy	Overall	Mutation (%)	Single nucleotide polymorphism (%)
Age					26 (11.9%)	11 (5.0%)
<65	55	27	8	90 (41.1%)	8 (8.9%)	5 (5.6%)
≥65	72	37	20	129 (58.9%)	18 (14.0%)	6 (4.7%)
Mean	65.2	64.7	66.6	65.5	67.4	69.8
Gender						
Male	86	28	20	134 (61.2%)	12 (9.0%)	7 (5.2%)
Female	41	36	8	85 (38.8%)	14 (16.5%)	4 (4.7%)
Race						
Caucasian	87	62	28	177 (80.8%)	25 (14.1%)	8 (4.5%)
AA	40	1	0	41 (18.7%)	1 (2.4%)	3 (7.3%)
Other	0	1	0	1 (0.5%)	0	0
Smoking						
Nonsmoker	13	14	7	34 (15.5%)	12 (35.3)*	1 (2.9%)
Former smoker	42	34	11	86 (39.4%)	11 (12.6%)	5 (5.7%)
Current smoker	72	16	10	98 (44.8%)	3 (3.1%)	5 (5.1%)
Pathology						
Adenocarcinoma	72	64	28	164 (74.9%)	25 (15.2%)†	10 (6.1%)
Squamous or other NSCLC	55	0	0	55 (25.1%)	1 (1.8%)	1 (1.8%)
Family cancer history	81/115	38/64	NA	118/179 (66%)	10/19 (52.6%)	8/11 (72.7%)
Mutation of *EGFR*	8 (6.3%)	13 (20.3%)	5 (17.9%)	26	$P = 0.011$‡	

NOTE. Percentages indicate the portion of the indicated change in overall samples.
*$P < 0.0001$.
†$P < 0.01$.
‡Distribution of mutation among the three cohorts.

Table 2 Characteristics of non–small cell lung patients with *EGFR* gene mutations

Patient no.	Ethnicity	Gender	Age	Smoking*	Pathology†	Stage	Mutation	Exon	Nucleotides	Amino acid
46317T(M)	Caucasian	F	83	NS	ADC	IIA	Point mutation	18	2,155 G→C	G719R
98A(M)	Mix-Caucasian	F	70	FS	ADC	IA	12-bp deletion (2,240-2,251)	19	TAAGAGAAGCAA	L747_751Tdel
114(M)	Caucasian	F	62	FS	ADC	IV	Deletion of 15 bp (2,235-2,249)	19	AATTAAGAGAAGCAA	E746_A751del
119A(M)	Caucasian	M	66	FS	ADC	IA				
46125T(M)	Caucasian	F	32	CS	ADC	IB				
11318T(H)	Caucasian	F	48	NS	SCC	II				
14753T(H)	Caucasian	F	62	NS	ADC	I				
0006T(I)	Caucasian	M	69	NS	ADC	IIB				
0059T(I)	Caucasian	M	67	FS	ADC	IIIA				
1064T(I)	Caucasian	F	71	NS	ADC	IB				
1501T(H)	Caucasian	F	63	NS	ADC	I	18-bp deletion (2,240-2,257)	19	TAAGAGAAG-CAACATCTC	L747_753Pdel
1650T(H)	AA	M	73	FS	ADC	II				
23(M)	Caucasian	F	42	FS	ADC	IIA	2-bp deletion (2,554-2,555)	20	AC	D770del
96A(M)	Caucasian	M	70	FS	ADC	IIB				
10419(M)	Caucasian	F	72	FS	ADC	I	Point mutation	20	2,563 A→T, 2,565 G→A	H772L, V773M
41905(M)	Caucasian	M	80	NS	ADC	IIIA	Point mutation	20	2,570 & 2,571 GG→TT	G778F
78519(M)‡	Caucasian	M	81	FS	ADC	IA				
136(M)	Caucasian	M	80	CS	ADC	IB	Point mutation	21	2,573 T→G	L858R
57768T(M)	Caucasian	F	80	NS	ADC	IIIA				
1186T(H)	Caucasian	M	73	NS	ADC	II				
11139T(H)	Caucasian	M	49	CS	ADC	I				
10198T(H)	Caucasian	F	62	NS	ADC	III				
15479T(H)	Caucasian	F	72	NS	ADC	I				
0146T(I)	Caucasian	M	71	FS	ADC	IIIA				
1119T(I)	Caucasian	F	74	NS	ADC	IIIA				
57767T(M)‡	Caucasian	M	82	FS	ADC	IV				

NOTE. Patient numbers are as indicated. The letter after each patient number indicates the source of the sample where H, University of Maryland; M, Mayo Clinic; I, U Milan, Italy.

*FS, former smoker; NS, nonsmoker; CS, current smoker.

†ADC, adenocarcinoma; SCC, squamous cell carcinoma.

‡Case 57767M and 78519M had homozygous mutation. Normal sample could not be amplified by PCR.

patients had *EGFR* gene mutations (14.1%; 95% CI, 9.4-20.1%) compared with only 1 of 41 patients of African American descent (2.4%; 95% CI, 0.0-12.9%, $P = 0.035$).

Similar to the previous reports (13–15), 25 of the 26 *EGFR* gene mutations were identified among the 164 adenocarcinoma (15.2%; 95% CI, 10.1-21.7%), whereas only one was observed in a squamous cell carcinoma (1 of 55, or 2.0%; 95% CI, 0.0-9.7%, $P = 0.008$). Patients who were nonsmokers were more likely to have the *EGFR* gene mutation, with 12 of 34 nonsmokers (35%) having mutations, compared with 3% and 13% in current and former smokers, respectively ($P < 0.0001$). Frequencies of mutations in former and current smokers were also statistically different ($P = 0.01$) with former smokers more likely to have tumors with an *EGFR* gene mutation than current smokers. Family histories of lung and other cancers were available from 179 patients. The frequency of *EGFR* mutations was statistically the same among individuals with a family history of lung or other cancer (10 of 118, 8.5%) and those with no family history of cancer (9 of 61, 14.8%; $P = 0.196$). When stages were considered, 9% of stage I had *EGFR* mutations, whereas 23% of stage II and 11% of stage III and IV tumors had *EGFR* gene mutation ($P = 0.082$).

The clinical characteristics of all patients and their molecular changes in the *EGFR* gene are summarized in Table 2. In total, we identified one case (46317M) with a point mutation in exon 18 that resulted in a substituted amino acid from glycine to arginine at codon 719 (G719R). Thirteen (13) tumors had deletions in exons 19 and 20. Among these cases, one had a 12-bp deletion (del L747_751T), eight had a 15-bp deletion (del E746_A751), two had an 18-bp deletion (del L747_753P) in exon 19, and two cases had a 2-bp deletion (del D770) in exon 20. In addition, two different types of mutations were observed in exon 20. One of these changes included a substitution of two adjacent nucleotides in sample 41905M resulting in a change of glycine to phenylalanine at codon 778 (G778F) and the other involved two amino acids at codons 772 and 773 (H772L, V773M) in one sample (10419M). In exon 21, a total of nine tumors had a substitution of a single nucleotide at amino acid codon 858, which resulted in a change of leucine to arginine (L858R). All mutations were heterozygous and not present in the paired normal tissues, except for case 57767M where the mutation seemed to be homozygous but the paired normal tissue could not be analyzed available to confirm whether the change was also present in the germ-line tissues.

In addition to the mutational changes described above, our study also observed 11 novel genomic variations in the *EGFR* gene (Table 3). We also observed two previously reported common polymorphisms that occur at nucleotide position 2,596 in exon 20 and at about 60 bp upstream of exon 20 (rs10241451). These single nucleotide polymorphisms were highly polymorphic and were observed in 161 and 78 cases, respectively, among the 219 tested samples. All variant

Table 3 Germ-line variations of *EGFR* gene in patients with non–small cell lung cancers

Patient no.	Ethnicity	Gender	Age	Pathology	Stage	Exon	Polymorphism	Nucleotide or amino acid
217A(M)	Caucasian	M	89	ADC	IA	19	Deletion of 22 (Intron 20)	TGCTGTGTGGGGGTCCATGGCT
85978(M)*	Caucasian	F	46	ADC	IV	20	2,604 C→T	T790M
11008(H)	AA	F	60	SCC	I	Intron 19	164,067 C→T	No change
1712(H)	Caucasian	M	74	ADC	I	Intron 20	164,315 A→C	No change
96A(M)	Caucasian	M	70	ADC	IIB	21	2,508 C→T	No change
41540T(M)	Caucasian	M	82	ADC†	IV			
76954T(M)	Caucasian	M	84	ADC†	IV			
1025T(H)	AA	M	63	SCC	I			
10403T(H)	AA	F	55	ADC	I			
11191T(H)	Caucasian	M	47	ADC	I			
1061T(I)*	Caucasian	F	68	ADC	IIB	21	L883V, H835L	2,497 T→G, 2,504 A→T

*Normal sample could not be amplified by PCR.
†Adenocarcinomas with bronchioloalveolar features.

changes listed in Table 3 were novel and observed at frequencies between 0.5% and 3%. None of the patients had a somatic *EGFR* gene mutation exception in case 96A in which the tumor sample from the patient also had a 2-bp deletion in exon 20 as well as a germ-line variation at exon 21. The novel polymorphic changes identified in this study included a 22-bp deletion beginning at 15 nucleotide 5′ of intron 20 observed in one patient; a silent nucleotide change at codon 2,508 in exon 21 identified in six cases; two noncoding region changes in introns 19 and 20, respectively. We also observed one patient (1061I) with two germ line variants in exon 21 resulting in amino acid codon changes of L883V and H835L and one patient (85978M) with a change of C to T at codon 790 that resulted in a change of threonine to methionine (T790M) in exon 20. The changes in samples 1061I and 85978M both seemed to be germ line based on the sequencing trace files in the tumor sample even though the paired normal tissues were not available for confirmation. Overall, the detected polymorphic variants were distributed similarly across the categories examined (Table 1). However, their occurrences were too low to determine whether there are any associations with epidemiologic, demographic, or clinical factors as well as *EGFR* gene mutations. Figure 1 summarizes the relative location and the occurrence of all somatic and germ-line alterations identified in the present study. In addition to the several novel somatic mutations identified here, the most common types of *EGFR* gene mutations appeared to be either a 12- to 18-bp deletion within exon 19 or a T to G transversion at nucleotide position 2,573 in exon 21, which jointly accounts for 20 of 26 (77%) mutations identified in our study and in 47 of 56 (84%) *EGFR* gene mutations reported to date (13–15).

Our data, derived from a diverse series of NCSLC patients, revealed that the frequency of the *EGFR* gene mutation is quite high (14.1%) among nonselected lung cancer patients who were Caucasians and nearly 18% for Caucasians with lung adenocarcinomas. In our study, only one tumor from 41 patients of African American descent (2.4%) had a tumor with *EGFR* gene mutations. This rate was statistically different from what was observed in the Caucasian population ($P = 0.03$) in our cohort and represents an odds ratio of 6.58 (exact CI, 1.01-276.8) for Caucasian patients. Our data also showed that patients with squamous cell lung cancers were much less likely to have tumors with the *EGFR* gene mutation (1 of 55, 1.8%). In contrast, although nonsmokers were more likely to have tumors with *EGFR* gene mutations, patients who are current or former smokers accounted for half of all patients whose tumors had the mutation. Furthermore, 46% of the cases with *EGFR*

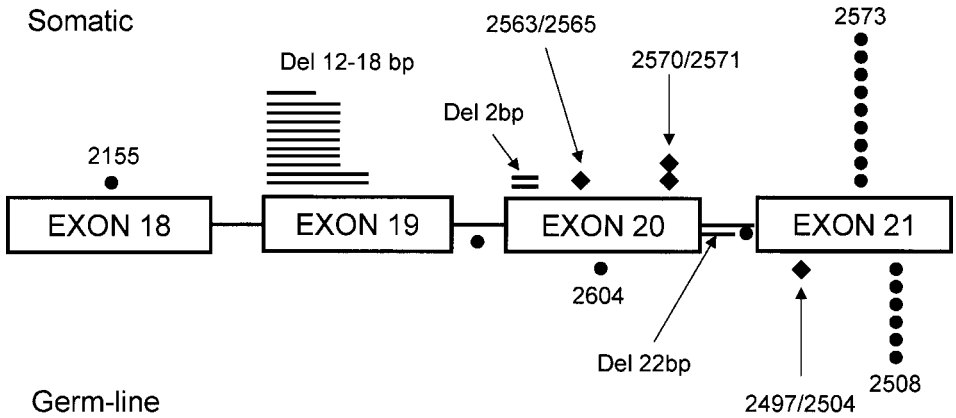

Fig. 1 Distribution and relative occurrence of *EGFR* gene mutations and germ-line variations in NSCLC. The kinase domains of the *EGFR* gene encompassing exons 18, 19, 20, and 21 are shown. The nucleotide positions of the genetic changes are as indicated. Mutations are shown above the exons and germ-line variations are shown below the respective exons. The relative lengths of exon 19 and 20 deletions, which varied from 2 to 24 bp, are represented by the relative length of the bars. Each symbol represents one case, where dots (●) represent samples with point mutations and diamonds (♦) represent those having changes involving two nucleotides.

gene mutations were men and the age of patients with the *EGFR* gene mutations spans from 32 to 83 years.

It is worth noting that our study involved a diverse set of population which comprised patients with varied demographical and epidemiologic characteristics. Therefore, the rate of *EGFR* gene mutation reported here might not be a true estimate of its prevalence in the population. For instance, when all patients were considered together, there was a significant difference in the rate of *EGFR* gene mutations in patients among the three cohorts (University of Maryland versus Mayo and Italy, $P = 0.01$, Table 1). However, the frequency of *EGFR* gene mutations was not statistically different with 11% from the University of Maryland, 18% from Italy, and 19% from the Mayo Clinic when only Caucasian patients with lung adenocarcinomas were considered ($P = 0.353$).

The potential for an effective therapeutic response for lung cancers with a mutated *EGFR* gene (13–15) makes it essential that appropriate patients be screened for the mutation. The results of our study show that the occurrence of the *EGFR* gene mutation is substantial (17.6%) in Caucasians with adenocarcinomas. Furthermore, our study also showed that *EGFR* mutations are quite common in men, as well as in women, and in individuals with a history of smoking, as well as nonsmokers. Given the estimated over 150,000 new patients who are diagnosed with lung cancer each year in the United States alone (1), the efficient screening of tumors from all lung cancer patients will potentially identify more than 18,000 patients whose tumors carry the *EGFR* gene mutation and who can benefit from the target specific therapy. The relatively specific and focused mutation hotspots thus far identified by us and others make it possible to develop rapid molecular detection assays (17) for the robust and sensitive detection of *EGFR* gene mutations.

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REFERENCES

1. Cancer Facts 2003. Atlanta (Georgia): American Cancer Society; 2003.

2. Parkin DM. Global cancer statistics in the year 2000. Lancet Oncol 2001;2:533–43.

3. Breathnach OS, Freidlin B, Conley B, et al. Twenty-two years of phase III trials for patients with advanced non–small-cell lung cancer: sobering results. J Clin Oncol 2001;19:1734–42.

4. Hirsch FR, Scagliotti GV, Langer CJ, Varella-Garcia M, Franklin WA. Epidermal growth factor family of receptors in preneoplasia and lung cancer: perspectives for targeted therapies. Lung Cancer 2003;41: S29–42.

5. Hunts J, Ueda M, Ozawa S, Abe O, Pastan I, Shimizu N. Hyperproduction and gene amplification of the epidermal growth factor receptor in squamous cell carcinomas. Jpn J Cancer Res 1985;76:663–6.

6. Rusch V, Klimstra D, Venkatraman E, Pisters PW, Langenfeld J, Dmitrovsky E. Overexpression of the epidermal growth factor receptor and its ligand transforming growth factor α is frequent in resectable non–small cell lung cancer but does not predict tumor progression. Clin Cancer Res 1997;3:515–22.

7. Wakeling AE, Guy SP, Woodburn JR, et al. ZD1839 (Iressa): an orally active inhibitor of epidermal growth factor signaling with potential for cancer therapy. Cancer Res 2002;62:5749–54.

8. Ciardiello F, De Vita F, Orditura M, Tortora G. The role of EGFR inhibitors in non–small cell lung cancer. Curr Opin Oncol 2004;16: 130–5.

9. Andratschke NH, Dittmann KH, Mason KA, et al. Epidermal growth factor receptor as a target to improve treatment of lung cancer. Clin Lung Cancer 2004;5:340–52.

10. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non–small-cell lung cancer. J Clin Oncol 2003;21: 2237–46.

11. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non–small cell lung cancer: a randomized trial. JAMA 2003;290:2149–58.

12. Janne PA, Gurubhagavatula S, Yeap BY, et al. Outcomes of patients with advanced non–small cell lung cancer treated with gefitinib (ZD1839, "Iressa") on an expanded access study. Lung Cancer 2004; 44:221–30.

13. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non–small cell lung cancer to gefitinib. N Engl J Med 2004;350: 2129–39.

14. Paez JG, Janne PA, Lee JC, et al. *EGFR* mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304: 1497–500.

15. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306–11.

16. Visbal AL, Williams BA, Nichols FC III, et al. Gender differences in non–small cell lung cancer survival: an analysis of 4,618 patients diagnosed between 1997 and 2002. Ann Thorac Surg 2004;78:209–15.

17. Dong SM, Traverso G, Johnson C, et al. Detecting colorectal cancer in stool with the use of multiple genetic targets. J Natl Cancer Inst 2001;93:858–65.